Author’s response to reviews

Title: The effect of diluting povidone-iodine on bacterial growth associated with speech

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Kemal Ornek (Reviewer 1): This is an interesting study reporting the effect of varying concentrations of povidone iodine on bacterial growth during speech. The authors conclude that lower than 5% povidone iodine may not be effective in reducing bacterial load. As mentioned in the discussion section, there are some limitations like identification of bacterial species, in vitro design etc. and they decrease the scientific value of this interesting research. I have still some concerns to be addressed:

1. Why did not the participants receive an upper tract sampling or nose and throat examination before study? What kind of similarity exists between ocular flora and upper tract flora, please give detailed information.

The participants did not receive any upper tract sampling or nose and throat examination before the study as we believe the hypothesis of the upper respiratory tract flora being dispersed onto the operative field is well established.

While the information from a nose and throat sample would be valuable to compare to the flora present on the culture plates, neither was completed as we were confident from the evidence in the literature that bacterial dispersal onto the injection surface via droplet spread was a significant factor for the disproportionate representation of streptococcus species in culture-positive endophthalmitis cases.

The study exclusion criteria prohibited patients with an active infection, or treatment for any active infection from taking part in the study.

2. Why did you choose only one type of agar?
Only 1 type of agar was chosen for consistency. Previous studies in the literature have used blood agar plates and we add to this literature with our study.

3. Why was the agar first coated with povidone iodine solution and later tested?

When an intravitreal injection is given, protocols suggested by RANZCO and other governing bodies emphasise the importance of asepsis with PI or chlorhexidine (for those allergic to PI) prior to the injection.

4. Did gender or age of the participants have an effect on the results of the study?

There were 6 males and 15 females. The mean age of the participants was 29.7 years (range 21-51). A sub-analysis of age / gender and CFU count was not conducted.

5. We know that ocular surface and eyelid flora are primarily related with endophthalmitis cases. Povidone iodine concentration of 5% seems to reduce speech related bacterial load and seems to be effective for this part of the procedure, so could it also be enough for the ocular flora? This is only the lowest concentration to be used.

While endophthalmitis cases post operatively have commonly been likely to staphylococcal species which predominate on the ocular surface,(1) Streptococcus species have been identified as significant causes of culture-positive endophthalmitis following intravitreal injections.(1-4) Studies have however shown streptococcus species to make up only a small portion of the normal conjunctival flora.(5-7) As such, 5% PI should provide adequate antisepsis against both ocular surface flora and oral flora that may be dispersed onto the ocular surface.

Hiroyuki Nakashizuka, Ph.D.,M.D. (Reviewer 2): Recently, the use of povidone-iodine as a strategy for endophthalmitis in increasing intravitreal injection is advocated and is attracting a great deal of attention. The author's experiments methods are clear but not realistic.

We also struggle to understand why they reproduced an environment that they talk for 5 minutes after the addition of povidone-iodine.

The time duration was chosen to maximize the potential bacterial dispersal onto the blood agar plates and by extension assess the effectiveness of the various PI concentrations under a heavy bacterial load. While this may be unrealistic, our aim was to assess the effectiveness of PI concentrations under a heavy bacterial load.

In the protocol of the intravitreal injection, the use of face mask or avoidance of talking is strongly recommended[1].
Despite the identification of streptococcus species as the causative organism in culture positive cases of endophthalmitis post intravitreal injections and both Wen et al(8) and Doshi et al(9) showing an effective reduction in CFUs on culture plates from bacterial dispersal associated with speech, some ophthalmologists continue to refrain from the regular use of surgical masks during intravitreal injections. Silence has similar effectiveness as wearing a face mask at reducing bacterial dispersion associated with speech. Schimel et al(10) argues that this be considered over surgical mask use but this may be impractical in a setting where communication is important.

Immediate after disinfection with povidone-iodine, intravitreal injections are commonly performed, and it cannot be assumed that speech should be continued for as long as 20 cm, even for 5 minutes after disinfection.

In reality the distance between patient and clinician may vary both within a given IVI episode and between clinicians. The time spent talking will also differ between patients and from clinician to clinician. Assistants will bring an additive effect. The distance and time duration was chosen to maximise the potential bacterial dispersal onto the blood agar plates and by extension assess the effectiveness of PI under a heavy bacterial load. Protocols recommended waiting 30 seconds after application of povidone iodine as this has been shown to be most effective at reducing the number of colonies cultured form the conjunctiva.(11)

The excess is discarded after 2.5 ml of povidone-iodine has been added to the blood agar test plate, but the protocol recommends that povidone-iodine should not be dried before intravitreal injection. Experiments performed without discarding the excess may have been more realistic.

Protocols recommended waiting 30 seconds after application of povidone iodine as this has been shown to be most effective at reducing the number of colonies cultured form the conjunctiva.(11) The excess was discarded in an attempt to mimic excess povidone-iodine on the ocular surface draining away from the ocular surface during the 30 second wait time.

It is necessary to state the size of the blood agar test plate, how the medium was supplemented with povidone-iodine, and how the excess was discarded.

The agar plates were 100 x15mm (diameter x height) in size. The surface of the agar plate was irrigated with PI in the same way the ocular surface is irrigated with PI prior to an intravitreal injection. Excess was discarded by inverting the agar plate to allow the excess to drain off. These changes have been made to the manuscript (methods section, line 141-142, page 6 and line 154-155, page 7)

In the discussion session, authors commented that though the free iodine becomes more abounding, when the concentration of the povidone iodine is low proportionally, there are the possibility in which it differs in vivo. However, there is a report that the endophthalmitis can be prevented using the povidone iodine of 0.25% in the intravitreal injection[2]. Even from this point of view, the bacterial load in this experiment may not be realistic.

Differing findings have been made regarding PI in vitro and in vivo and this is commented on in the discussion. While the article referred to by Shimada et al(12) found no cases of
endophthalmitis when facemasks were worn and 0.25% PI was used for antisepsis prior to an IVI; guidelines recommend irrigation with 5-10% PI prior to IVI. As commented on in the discussion, In a prospective, blinded study, Ferguson et al(13) found 5% PI to be more effective than 1% PI in decreasing the human conjunctival bacterial flora in pre-operative cataract surgery.